Phytotaxa 297 (2): 139–156
http://www.mapress.com/j/pt/
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Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.297.2.2

Speciation in the genera *Anthericum* and *Chlorophytum* (Asparagaceae) in Ethiopia—a molecular phylogenetic approach

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Abstract

Sister group relations of Ethiopian species of *Anthericum* and *Chlorophytum* and variation patterns in the *C. gallabatense* and *C. comosum* complexes were studied using molecular phylogenetic analyses, morphometrics, and scanning electron microscopy of seed surfaces. Results indicate that molecular data largely support previous morphological conclusions, and that speciation has occurred in Ethiopia at least three times in *Anthericum* and repeatedly within different subclades of *Chlorophytum*. Areas particularly rich in endemic species are the lowland area around Bale Mountains in SE Ethiopia and in the Beninshangul Gumuz regional state in W Ethiopia near the border to Sudan. A new species, *Chlorophytum mamillatum* Elden & Nordal, is described, and the names *C. tordense* and *C. tetraphyllum* are re-instated.

Key words: Anthericaceae, endemism, Horn of Africa, sister group relations, taxonomy

Introduction

The family Anthericaceae was revised for the Flora of Ethiopia and Eritrea (FEE) by Nordal (1997); different representatives are shown in Fig. 1 A–I. In APG III (2009), Anthericaceae is sunk into a broadly-defined Asparagaceae. In FEE, three species of *Anthericum* Linnaeus (1753: 310) and 23 species of *Chlorophytum* Ker Gawler (1807: 1071) were recorded, of which two and eight, respectively, are endemic to the Horn of Africa, including NE Kenya (Fig. 2A). Furthermore, there is a Somalian endemic element of seven species of *Chlorophytum* (Nordal & Thulin 1993; Thulin 1995; Nordal *et al.* 2001). Some unsolved problems in species delimitation were pointed out in the FEE, for example that "shade forms" of *C. gallabatense* Schweinfurth ex Baker (1876: 325) (Fig. 1H) might be confused with *C. comosum* (Thunberg 1794: 63) Jacques (1862: 345) because of the almost prostrate inflorescences in both. The two were, however, suggested to be distinguished on flower colour (greenish in the former and whitish in the latter) and on roots (lateral tubers in the former and subterminal tubers in the latter). This problem of species delimitation has so far not been solved.

Since 1997, the following three new endemic species of *Chlorophytum* have been described from Ethiopia: *C. herrmannii* Nordal & Sebsebe (2005: 326), *C. serpens* Sebsebe & Nordal (2005: 328), and *C. pseudocaule* Tesfaye & Nordal (2007: 129) (Fig. 1E). Further, the endemic *C. neghellense* Cufodontis (1939: 311) (Fig 1B) was transferred to *Anthericum* as *A. neghellense* (Cufodontis) Bjorå & Sebsebe (in Bjorå 2008: 122). In the current understanding of *Anthericum*, the genus consists of seven species, four of which are found in Ethiopia. *Chlorophytum*, on the other hand, includes around 180 species with 25 occurring in Ethiopia.

The genera *Anthericum* and *Chlorophytum* have recently been subjected to preliminary molecular phylogenetic investigations (Hoell 2005; Bjorå 2008; Elden 2010). Bjorå (2008) showed that specimens of *C. comosum* from southern Africa, where the type specimen was collected, did not group with "*C. comosum*" *sensu* Flora of Tropical East Africa (FTEA, Nordal *et al.* 1997).

Accepted by Abraham van Wyk: 30 Jan. 2017; published: 27 Feb. 2017

Our primary aims with the present paper are:

1. To investigate the phylogenetic sister group relations of the Ethiopian species of *Anthericum* and *Chlorophytum* to elucidate possible speciation patterns.
2. To assess the variation patterns in the *C. gallabatense* and *C. comosum* species complexes and establish if the so-called "shade form" of *C. gallabatense* mentioned in FFE should be recognized taxonomically.

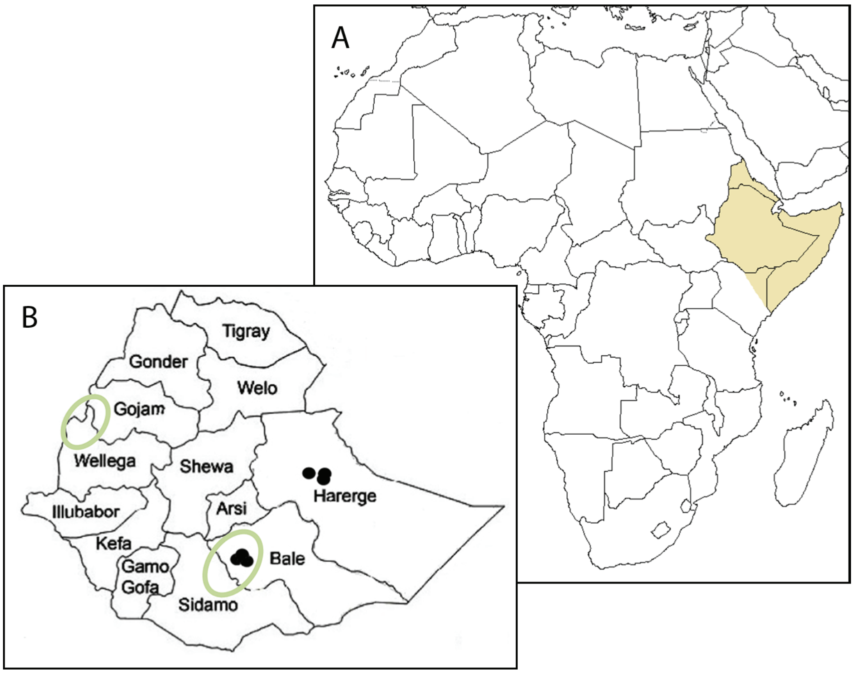


FIGURE 1. A. Map of Africa; Horn of Africa, including north-eastern Kenya, highlighted. B. Map of floristic regions in Ethiopia; dots displaying the distribution of *Chlorophytum mamillatum* ("shade form" of *C. gallabatense* in FFE). Green circles indicate the two main evolutionary hotspot areas of *Chlorophytum*.

Materials and Methods

Plant material for molecular work

Plant materials used in the present study are from herbarium specimens held at BRLU, ETH, K, O, and WAG, and from silica-dried leaf samples collected during field work in Ethiopia in 2007 (vouchers deposited at ETH). A total of 69 specimens (Table 1) were studied. Available specimens from Ethiopia were supplemented by specimens from other parts of Africa to attain a broader taxonomic and geographical context. Unfortunately, two relevant species, *C. inconspicuum* (Baker 1877: 71) Nordal (1993: 63) and *C. bifolium* Dammer (1905: 66), could not be included in the present study. Both species are very rare, and may have gone extinct. The species concepts followed herein are according to FTEA, FEE, and Flora Zambesiaca (Kativu *et al.* 2008). There is one discrepancy in these floras; *Chlorophytum tordense* Chiovenda (1916: 173) of FEE was reduced to *C. affine* Baker (1875: 160) var. *curviscapum* (Poellnitz 1942: 122) Hanid (1974: 588) in FTEA.

DNA extraction, PCR amplification and DNA sequencing

Total genomic DNA was extracted from herbarium specimens or silica-dried leaf samples using the DNeasy Plant mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. We PCR amplified and sequenced one nuclear ribosomal (ITS1 = nuclear ribosomal internal transcribed spacer 1) and two plastid (*trn*L-F, *rps*16 intron) DNA regions. For amplifying the ITS1, we used modified versions of the primers ITS5 and ITS2 (White *et al.* 1990); ITS5mod: 5'GGAAGTAAAAGTCGTAACAAGG'3 and ITS2mod: 5'GCTACGTTCTTCATCGATGC'3. We used the e and f primers of Taberlet *et al.* (1991) and the rpsF and rpsR2R primers of Oxelman *et al.* (1997) to PCR amplify the *trn*L-F spacer and the *rps*16 intron, respectively.

The genetic regions were amplified from 2 µL unquantified genomic DNA in 25 µL reactions using either the

AmpliTaq DNA polymerase buffer II kit (Applied Biosystems, Foster City, CA, USA) containing 0.2 mM of each dNTP, 0.04 % bovine serum albumen (BSA), 0.01 mM tetramethylammonium chloride (TMACl), and 0.4 µM of each primer, or the Illustra™ puReTaq Ready-To-Go PCR Beads (GE Healthcare, UK) with 1.5 µL (5 µM) of each primer and 20 µL milliQ H_2O. We performed all amplifications on an Eppendorf Mastercycler EP gradient S under the following cycling conditions: 94°C for 2.5 min, 32 cycles of 94°C for 30 s, 53°C for 30 s, 72°C for 50 s, followed by 72°C for 4 min. We purified the PCR products using 2 µL 10 times diluted ExoSAP-IT (USB Corporation, Cleveland, OH, USA) to 5 µL PCR product, incubated at 37°C for 30 min followed by 15 min at 80°C. Prepared amplicons for sequencing, contained 1 µL purified PCR product, 1 µL of 10 µM primer (the same primer as used for the PCR), and 8 µL milliQ H_2O. After sequencing using the ABI BigDye Terminator sequencing buffer and the v3.1 Cycle Sequencing kit (Applied Biosystems), the sequences were processed on an ABI 3730 DNA analyser (Applied Biosystems). We assembled and edited the sequences using the ContiqExpress module in Vector NTI Advance™ 11.0 (Invitrogen Corporation, CA, USA). A total of 137 new sequences were generated for the present study (Table 1).

TABLE 1. List with voucher information (taxon name, herbarium, voucher identification and country of origin) and GenBank accession numbers for DNA sequences used in the present study. Sequences generated for the present have accession numbers beginning with KU880. Abbreviations: *A.* = *Anthericum*; *C.* = *Chlorophytum*; Herb. = voucher-holding herbarium; n/a = not available.

Taxon/Specimen No.	Herb.	Voucher ID	Locality	ITS1	*trn*L-F	*rps*16
Anthericum angustifolium Hochst. (1)	ETH, O	Sebsebe 4670	Ethiopia, Shewa, Changal area	KU880773	KU880872	KU880818
A. angustifolium (2)	ETH	Edwards *et al.* 5041	Ethiopia, N slopes of Alagi Mts in Tigray	KU880774	KU880873	KU880819
A. corymbosum Baker (1)	O	Nordal 2276	Ethiopia, Bale: 38 km E of Robe	KU880775	KU880874	KU880820
A. corymbosum (2)	ETH	Nordal 4601	Kenya, K4, Mua Hills, SW of Nairobi	EU128949	EU128939	EU128959
A. jamesii Baker	ETH	Melaku & Kalaeb 229	Ethiopia, c. 10 km f the turn to El Siro wells	KU880776	KU880875	KU880821
A. neghellense (Cufod.) Bjorå & Sebsebe	ETH, O	Nordal *et al.* 2218	Ethiopia, Sidamo: 70 km S of Agere Maryam	KU880777	KU880876	KU880822
A. ramosum L. (1)	O	Bjorå 855	Switzerland, Berner Oberland, Schrändli	KU880778	KU880877	KU880823
A. ramosum (2)	O	1968-810-S	Cult. Sweden, Stemåsa, Øland	KU880779	KU880878	KU880824
A. sp.	ETH	Sebsebe 6743	Ethiopia, Bale: Mega	KU880780	KU880879	KU880825
Agave chrysantha Peebles.	O	92-207S	Cult.	KU880782	KU880881	KU880827
A. sp.	O	96-195S	Cult.	KU880781	KU880880	KU880826
C. affine Baker var. *curviscapum* (Poelln.) Hanid (1)	ETH	Nordal *et al.* 2289	Ethiopia, Bale: Sof Omar	KU880783	n/a	KU880828
C. affine var. *curviscapum* (2)	ETH	Sebsebe *et al.* 4274	Ethiopia, Bale: Sof Omar	KU880784	n/a	KU880829
C. affine var. *affine* (3)	O	Nordal & Bjorå 4552	Zambia N: Ntumbachusi falls	EF999985	EU000019	KU880830
C. africanum Engl. var. *africanum*	O	A. Bjørnstad 2054	Tanzania T7: Mbeya D., Magangwe	EF999986	EU000020	n/a
C. africanum var. silvaticum (Dammer) Meerts	O	Nordal & Bjorå 4621	Kenya K3: Near Gilgil	EU000008	EU000041	EU128980
C. andongense Baker	O	Nordal & Bjorå 5013	Tanzania T3: Pare D., near Lembeni	EU128950	EU128940	EU128960
C. blepharophyllum Schweinf. ex Baker (1)	O	Hoell & Nordal 24	Zambia, B: Lukulu road	EF999989	EU000023	EU128961
C. blepharophyllum (2)	O	Hoell & Nordal 94	Zambia N, Lumangwe Falls	KU880785	KU880882	KU880832
C. blepharophyllum (3)	ETH	Tesfaye 1722	Ethiopia, Benishangul-Gumuz Region	KU880786	KU880883	KU880833
C. cameronii (Baker) Kativu (1)	K, BR	Reekmans 3955	Burundi, Bubanza, Gihungwe	KU880787	KU880884	n/a
C. cameronii (2)	ETH	Sebsebe *et al.* 6093	Ethiopia, Benshangul-Gumuz, Gojam	KU880788	KU880885	KU880834
C. colubrinum (1)	O	Nordal & Bjorå 4535	Zambia C: Kasanka	EF999991	EU000025	KU880835
C. colubrinum (2)	O	Mitchel 35	Zambia: Sientambo, Kalome	EF999990	EU000024	KU880836
C. comosum (Thunb.) Jacques (1)	O	Bjorå 703	Tanzania T2: Kilimanjaro, Umwe route	KU880789	KU880886	KU880837
C. comosum (2)	O	Bjorå 869	Tanzania T2: Pare Mts	KU880790	KU880887	KU880838
C. comosum (3)	O	Bjorå 870	Tanzania T2: Pare Mts	KU880791	KU880888	KU880839
C. comosum (4)	O	Hemp 3690	Tanzania T2: Kilimanjaro, forest Old Moshi	EU128952	EU128942	EU128964
C. comosum (5)	O	Nordal 3162	Zimbabwe, Cult. in Harare	EF999993	EU000027	KU880840
C. comosum (6)	O	Nordal 3803	South Africa: Cape, Grootvatersbosch	EF999992	EU000026	EU128962
C. ducis-aprutii Chiov.(1)	ETH	Elden, Tesfaye & Nordal 1	Ethiopia, betw Robe and Sof Omar	KU880792	KU880889	KU880841
C. ducis-aprutii (2)	ETH	Tesfaye 1783	Ethiopia, Bale	KU880793	KU880890	KU880842
C. filipendulum Baker (1)	O	Nordal 3219	Zimbabwe E: Chipinge D., Kiledo lodge	EU128956	EU128944	EU128969
C. filipendulum (2)	O	Poulsen 956	Uganda U2: Masindi D. Budongo F.Res.	EF999994	EU000028	EU128968

...continued on the next page

TABLE 1. (Continued)

Taxon/Specimen No.	Herb.	Voucher ID	Locality	ITS1	*trn*L-F	*rps*16
C. gallabatense Schweinf. ex Baker (1)	O	Elden, Tesfaye & Nordal 7	Ethiopia, Sof Omar	KU880794	KU880891	KU880843
C. gallabatense (2)	O	Elden, Tesfaye & Nordal 8	Ethiopia, Sof Omar	KU880795	KU880892	KU880844
C. gallabatense (3)	ETH	Nordal 2225	Ethiopia, Shewa: 39 km S of Agere Maryam	KU880796	KU880893	KU880845
C. gallabatense (4)	O	Nordal 4560	Zambia N: E of Mununga	KU880797	KU880894	KU880846
C. cf. gallabatense (5)	O	Hoell & Nordal 25	Zambia B: Lukulu road	EF999996	EU000030	EU128971
C. geophilum Peter ex Poelln. (1)	ETH	Herrmann 36	Ethiopia, Benshangul-Gumuz, Gojam	KU880798	n/a	KU880847
C. geophilum (2)	O	Hoell & Nordal 26	Zambia B: Lukulu road	EF999998	EU000032	EU128972
C. herrmannii (1) Nordal & Sebsebe	ETH	Tesfaye *et al.* 1706	Ethiopia, Benshangul-Gumuz Region	KU880799	KU880895	KU880848
C. herrmannii (2)	ETH	Tesfaye 1276	Ethiopia, Benishangul Gumuz Region	KU880800	KU880896	KU880849
C. humifusum Cufod.	ETH, O	Nordal 2251	Ethiopia, Sidamo, near Neghelle	KU880801	n/a	n/a
C. lancifolium Welw. ex Baker	O	Nordal 4576	Zambia N: Kundabwika Falls	EU128957	EU128945	EU128973
C. longifolium Schweinf.	O	Nordal 1507	Zimbabwe S: Maswingo, near Great Zimbabwe	EU000001	EU000034	KU880851
C. macrophyllum Asch. (1)	ETH	Herrmann 102	Ethiopia, Wellega, Tsheborona	KU880802	KU880898	KU880852
C. macrophyllum (2)	O	Nordal 1033	Ethiopia: 51 km E of Nekemte	EU000002	EU000035	EU128974
C. macrosporum Baker	O, SRGH	Kativu 255	Zimbabwe C: Chegutu	EU000004	EU000037	KU880853
C. mamillatum sp. nov.	ETH, O	Elden, Tesfaye & Nordal 9	Ethiopia, Welmel River, Fenkel Kebale	KU880810	KU880906	KU880862
C. mamillatum sp. nov.	ETH					
C. pendulum Nordal & Thulin (1)	ETH	Nordal *et al.* 2260	Ethiopia, Sidamo: Mega	KU880803	KU880899	KU880854
C. pendulum (2)	O	Nordal *et al.* 2294	Ethiopia: Bale Region, 36 km S of Ginir	KU880804	KU880900	KU880855
C. polystachys Baker	O	Hoell & Nordal 7	Zambia S: S of Zimba, Monachongwe farm	EU000006	EU000039	KU880856
C. pseudocaule Tesfaye & Nordal	ETH	Tesfaye 1731	Ethiopia, Wellega, 15 km E of Asosa	KU880805	KU880901	KU880857
C. pterocarpum Nordal & Thulin	O	Nordal *et al.* 2288	Ethiopia, Bale: Sof Omar	KU880806	KU880902	KU880858
C. pubiflorum Baker	O	Nordal 4561	Zambia N: E of Mununga Bridge	KU880807	KU880903	KU880859
C. rubribracteatum (De Wild) Kativu	O	Bjorå 657	Zambia C: Chipata	KU880808	KU880904	KU880860
C. somaliense Baker	ETH	Haugen 1763	Ethiopia, Sidamo, Dollo area	KU880809	KU880905	KU880861
C. sparsiflorum Baker (1)	WAG	Wieringa *et al.* 5921	Cameroon, Littoral Province, Bekob camp	KU880812	n/a	KU880864
C. sparsiflorum (2)	WAG	Wieringa *et al.* 6452	Gabon, Haut-Ogooué, close to Akiéni	KU880813	n/a	KU880865
C. sphachelatum (Baker) Kativu	O	Hoell & Nordal 2	Zambia S: S of Zimba, Monachongwe farm	EU000009	EU000042	KU880866
C. subpetiolatum (Baker) Kativu (1)	O	Hoell & Nordal 15	Zambia B: Road to Mouyo	EU000011	EU000044	KU880867
C. subpetiolatum (2)	ETH	Herrmann 206	Ethiopia, Gojam: Assosa	KU880814	KU880908	n/a
C. suffruticosum Baker	O	A. Bjørnstad 2804	Kenya K7: Teita Dist, 51 km NW of Voi	EU000010	EU000043	KU880868
C. tetraphyllum Baker (1)	ETH	Ensermu & Lemessa 3503	Ethiopia, Shewa: 3 km W of Addis Abeba	KU880815	KU880909	KU880869
C. tetraphyllum (2)	ETH	Nordal 1030	Ethiopia, Shewa: betw. Gedo and Fincha	KU880816	KU880910	KU880870
C. viridescens Engl.	O	Nordal & Bjorå 5012	Tanzania T2: Moshi-Arusha Rd.	EU000012	EU000045	EU128981
C. zavattarii (Cufod.) Nordal	ETH	Nordal *et al.* 2281	Ethiopia, Bale: 12 km E of Goro	KU880817	KU880911	KU880871

Alignment and phylogeny reconstructions

Sequences from 69 accessions were manually aligned using BioEdit 7.0.9.0 (Hall 1999) and insertions/deletions (indels) were coded as present/absent and added to the matrices as additional, unordered characters using the program SeqState (Müller 2005) following the simple indel coding of Simmons & Ochoterena (2000). The data were analysed using maximum parsimony and Bayesian inference phylogenetic methods. Maximum parsimony analyses were performed using NONA (Goloboff 1999) in combination with WinClada v. 1.0 (Nixon 1999–2002) applying the heuristic search option with 2000 replicates and maxtrees set to 10 000, and otherwise default settings. Parsimony jack-knifing was undertaken with 1000 replicates and otherwise default setting. To check for gene tree incongruence, we compared by eye the strict- and jack-knife consensus phylogenetic trees of the three genetic regions separately. For the Bayesian phylogenetic analyses, MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) was used with prior models of nucleotide substitution set according to the output of MrModeltest (Nylander 2004). Posterior probabilities were determined by running one cold and three heated chains for 4 million generations in parallel mode, saving trees every 1000th generation. The analyses were performed twice to check their convergence for the same topology. For the Markov Chain to have converged, the average standard deviation of split frequencies (ASDSF) should fall below 0.01 when comparing two independent runs. We discarded as burn-in 25 % of the sampled trees, or the generations prior to the point where the ASDSF fell below 0.01 if passing the 25 % limit. We summarized the remaining trees as a 50 % majority rule consensus topology with Bayesian posterior probabilities (PP) of at least 0.9 and parsimony jack-knife (JK) support of at least 50 %. The preliminary parsimony consensus trees, from separate analyses of the three genetic regions, showed that only the plastid regions were congruent, although resolved to different extents and in different parts of the trees. Therefore, we established two datasets for the final analyses: (1) ITS1 with 69 accessions; and, (2) plastid DNA (pDNA) with 64 accessions. The Lifeportal server, University of Oslo, Norway (http://www.lifeportal.uio.no/root) was used for the model testing and Bayesian analyses.

Morphometry and scanning electron microscopy

We scored relevant morphological characters on 47 specimens of *Chlorophytum* sp. (= "shade form" of *C. gallabatense*), *C. gallabatense,* and *C. comosum* s.l., all deposited in ETH, K and/or O. Seed surfaces (testa ornamentation) of 17 specimens were investigated and photographed by use of a scanning electron microscope (SEM; Hitachi High Technologies and FE-PC-SEM software) after sputter coating with gold palladium.

Results

Alignments and phylogenetic analyses

The lengths in base pairs of the aligned genetic regions were: ITS1 317; *rps*16 855; and, *trn*L-F 268. The following numbers of indels were coded: ITS1 72; *rps*16 61; and, *trn*L-F 28. The estimated best fit models of nucleotide substitution were: GTR+G for ITS1 and *rps*16 ; and, HKY+G for *trn*L-F. Jackknife consensus trees with and without simple indel-coding were congruent. As the former had an overall higher resolution and branch support, all results presented herein are based on the indel-coded analyses.

TABLE 2. Morphological traits score for 47 specimens of *Chlorophytum mamillatum* (= "shade form" of *C. gallabatense*), *C. gallabatense* and *C. comosum* s.l. (see Table 1). The quantitative measures are given by range/mean.

	N=	No of branches	Peduncle erect/lax	Rhachis scabrid	Pedicel length (mm)	Ratio above/ below joint	Pedicel papillose	Flower colour	Capsule papillose	Root tubers on lateral branches
C. mamillatum	4	4–7/5	lax	+	5–7/5.7	0.4–0.6/0.5	+	white	+	–
C. gallabatense	10	2–12/5.1	erect	+	3–6/4.4	0.2–0.4/0.3	+	green	slight	+
C. comosum (S.Afr.)	20	1–4/1.6	lax	– (+)	4–14/8.9	0.2–0.5/0.4	– (+)	white	–	–
C. comosum (*sensu* FTEA)	13	1–2/1.1	lax	+ (–)	4–12/7.2	0.2–0.5/0.4	– (+)	white	–	–

Tree statistics from the maximum parsimony analyses of the two datasets were: (1) ITS1 – 32 most parsimonious trees (MPTs) of length 814 and with rescaled consistency index (RC) = 0.37 and homoplasy index (HI) = 0.50; and, (2) pDNA – 444 MPTs of length 2268 and with RC = 0.6 and HI = 0.28. In the Bayesian analysis of the ITS1 and pDNA datasets, the standard deviation of split frequencies (ASDSF) had fallen to 0.005877 and 0.009714 respectively, at termination, and the first 1000 generations (25 %) were discarded as burn-in.

The plastid regions separately rendered congruent topologies (not shown). Also the parsimony vs. the Bayesian analyses of all datasets were congruent, but resolved to different extents (not shown). The ITS1 vs. pDNA topologies were not congruent (Fig. 3a, b; clades discussed in the following are indicated with corresponding capital letters). One accession, *C. zavattarii* (Cufodontis 1939: 308) Nordal (1993: 65), included a high number of autapomorphic characters in the ITS1 alignment and attained incongruent positions in the ITS1 (clade E) vs. pDNA trees (clade F). Also two accessions of *C. geophilum* Peter *ex* Poellnitz (1943: 127) (Fig. 1G) attained incongruent positions (clade J vs. clade H). Apart from these incongruent patterns, the ITS1 and pDNA topologies supported by JK of at least 50 % or PP of at least 0.9 were congruent, but resolved to different extents and in different parts of the trees (Fig. 3a, b). The genus *Chlorophytum* is monophyletic with low support in the plastid tree (Fig. 3b) and unresolved with respect to *Anthericum* in the ITS tree (Fig. 3a). *Anthericum* is paraphyletic in the plastid tree (Fig. 3b) and unresolved with respect to *Chlorophytum* in the ITS tree (Fig. 3a). Monophyly was supported for most species with multiple accessions included, except for a few, where rather a geographic pattern among accessions was evident (e.g. *C. affine* and *C. comosum*). The two accessions of *C. affine* var. *curviscapum* constitute a highly supported subclade (ITS; Posterior probabilities (PP) 1/Jackknife branch support (JK) 100, pDNA; PP 1/ JK 99) with *C. humifusum* Cufodontis (1939: 311) as its closest sister. *Chlorophytum affine* var. *affine* together with *C. pubiflorum* and *C. polystachys* forms a sister clade (PP 0.9) to the

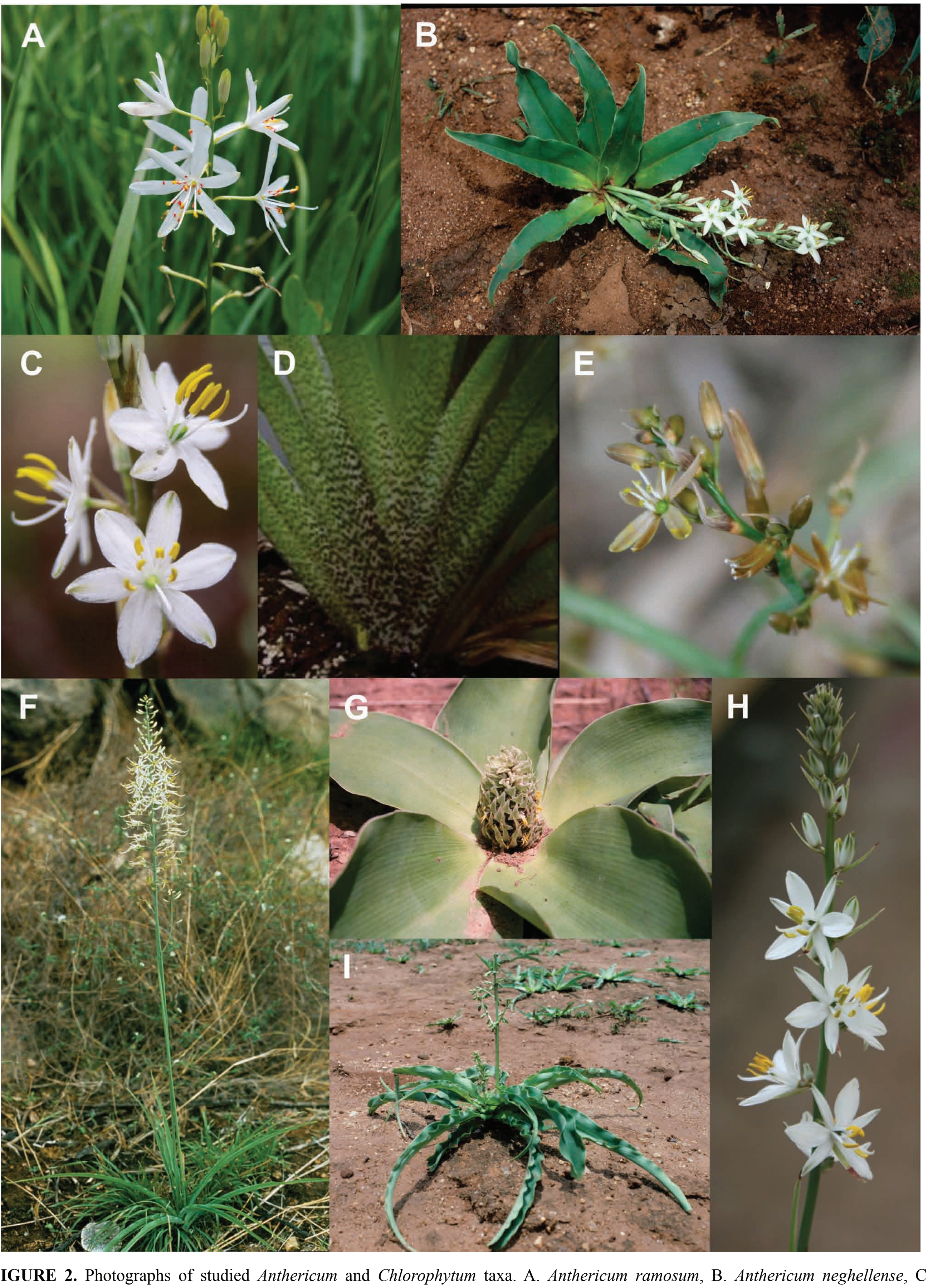
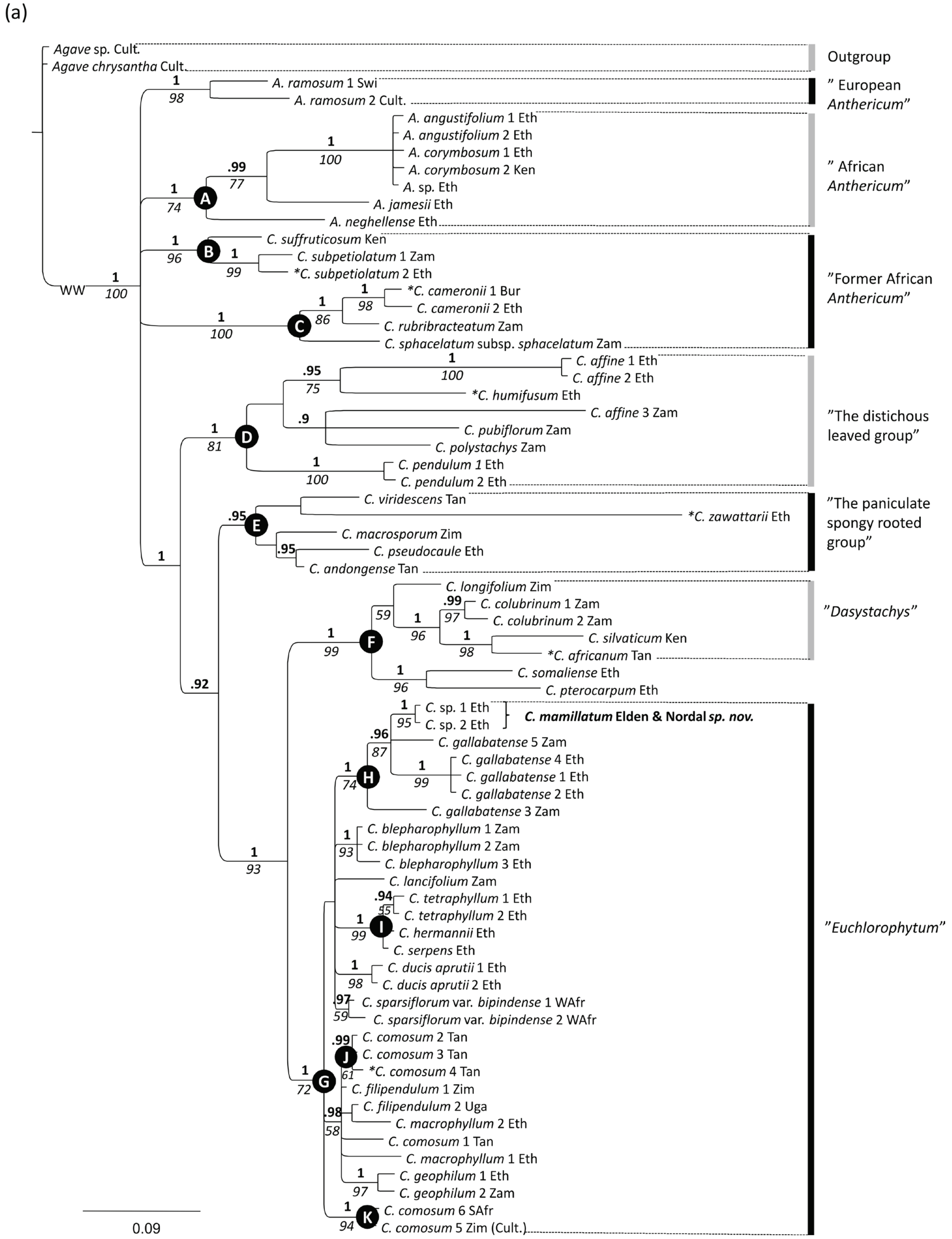


FIGURE 2. Photographs of studied *Anthericum* and *Chlorophytum* taxa. A. *Anthericum ramosum*, B. *Anthericum neghellense*, C. *Chlorophytum subpetiolatum*, D. *Chlorophytum affine* var. *affine*, E. *Chlorophytum pseudocaule*, F. *Chlorophytum somaliense*, G. *Chlorophytum geophilum*, H. *Chlorophytum gallabatense*, I. *Chlorophytum ducis-aprutii.* Photographs: Charlotte S. Bjorå (A), Inger Nordal (B, H, I), Gry S. Hoell (C, D, G), Tesfaye Awas (E), Mike Gilbert (F), Sebsebe Demissew (H).

C. affine var. *curviscapum* clade. *Chlorophytum comosum* is not monophyletic. The two accessions from southern Africa constitute clade K, with support 1 (PP) and 94 (JK) in the ITS analysis and support 0.97 (PP) and 71 (JK) in the pDNA analysis. Within clade J, the Tanzanian accessions of *C. comosum* are found. In the pDNA tree, they form a clade (PP 0.97/JK 88), while in the ITS tree, three accessions form a clade (PP 0.99) and one accession is unresolved.



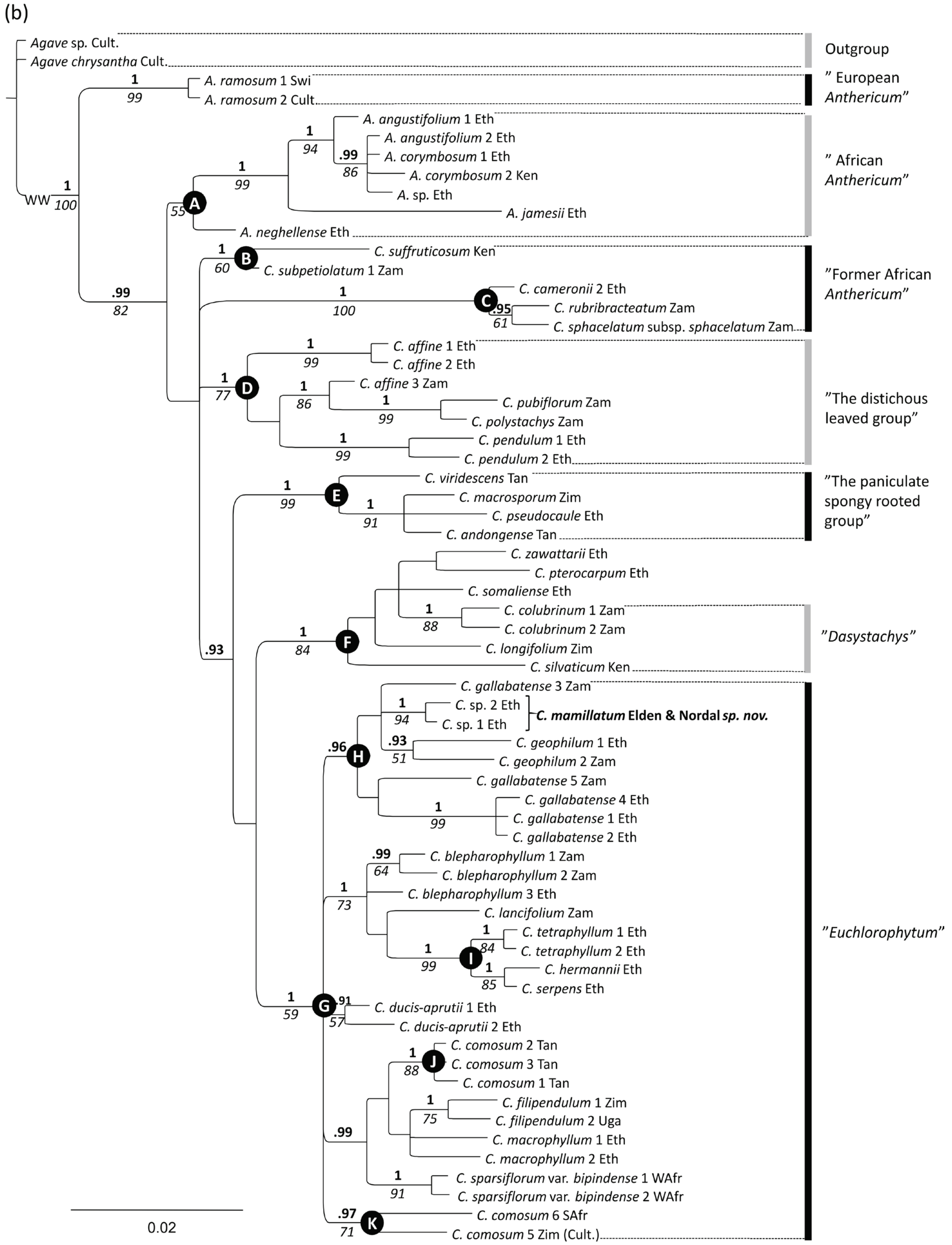


FIGURE. 3. The 50 % majority rule consensus phylograms for members of *Anthericum* and *Chlorophytum* from Bayesian analyses of (**a**) the ITS1 matrix with 69 acessions and 389 characters (incl. 72 coded indels), and (**b**) the concatenated matrix of two plastid (*rps*16 and *trn*L-F) DNA regions, 64 accessions and 1230 characters (incl. 89 coded indels). The Bayesian posterior probability values (PP) of at least 0.9 are reported in bold above branches, whereas maximum parsimony jack-knife support (JK) of at least 50 % are reported in italics below branches. Multiple accessions of the same species are numbered according to Table 1. "Morphological" groups (following Bjorå 2008) are indicated with bars to the right. Abbreviations: *A.* = *Anthericum*, Bur = Burundi, *C.* = *Chlorophytum*, Cam = Cameroon, Cult. = Cultivated, Eth = Ethiopia, Gab = Gabon, Ken = Kenya, SAfr = South Africa, Swi = Switzerland, Tan = Tanzania, WAfr = West Africa, Uga = Uganda, Zam = Zambia, Zim = Zimbabwe. The clades discussed in the text are marked with capital letters. The zigzag branch in each tree represents a manual shortening of long branches to reduce the size of a broad figure. Accessions only present in the ITS1 tree are indicated with an asterisk.

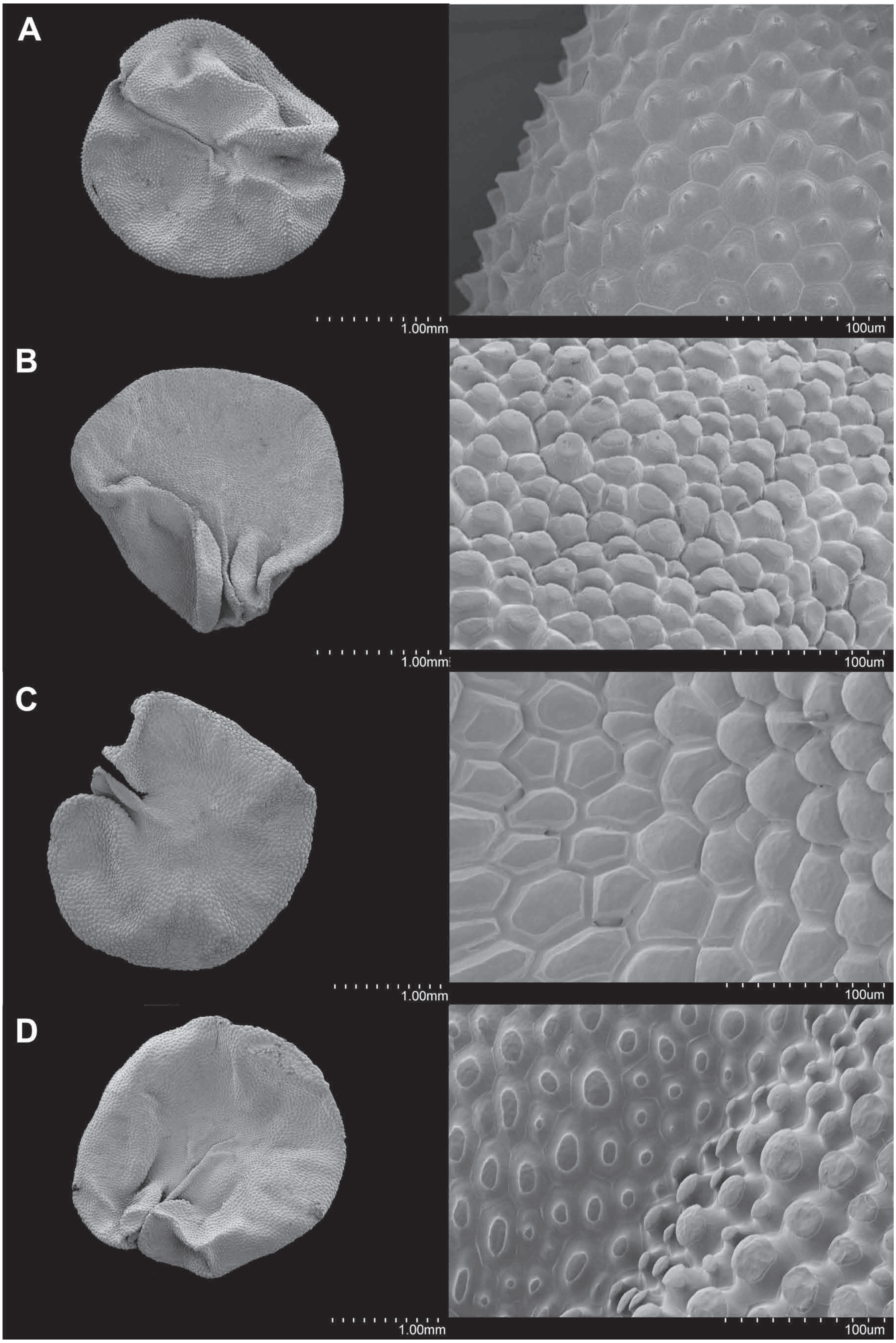


FIGURE. 4. Scanning electron micrographs of testa surface ornamentation in members of *Chlorophytum*. A. *Chlorophytum mamillatum* (= “shade form” of *C. gallabatense*), from Ethiopia, *Elden et al. 9* (O), B. *Chlorophytum comosum* from Malawi, *Brummitt & Banda 9846* (K), C. *Chlorophytum comosum* sensu FTEA from Uganda, *Lye 5536* (O). D. *Chlorophytum gallabatense* from Ethiopia, *Nordal et al. 2225* (O).

Morphometry and scanning electron microscopy

The putative undescribed taxon, *Chlorophytum* sp. (= "shade form" of *C. gallabatense*), shares morphological traits both with *C. gallabatense* and *C. comosum* s.l. (Table 2). It differs from *C. gallabatense* by its lax, prostrate inflorescence (the latter having stout, erect peduncles and ballistic seed dispersal), by the pedicel joint being in a more or less middle position (not above the middle), the white (not green) flowers, and by having the root tubers along the main axes of the roots (not on lateral branches). *Chlorophytum* sp. differs from *C. comosum* by having branched inflorescences, slightly papillose capsules and lacking pseudovivipary.

Seeds of *Chlorophytum* sp., *C. comosum* and *C. gallabatense* are relatively flat (Fig. 4A–D), although with some irregular folding mainly around the area of the micropyle/hilum. The seeds of *Chlorophytum* sp. appear to display slightly more folding than the others. The outline in surface view of the testa cells are rounded to slightly angular for all studied specimens. The periclinal epidermal cell walls are somewhat raised and rounded for all but *Chlorophytum* sp., which has periclinal walls that are conical and outdrawn to a distinct papilla.

Discussion

Phylogeny and speciation

Reciprocal monophyly of the two genera *Anthericum* and *Chlorophytum* is not supported by our molecular data (Fig. 3a, b), corroborating previous findings with less data (Hoell 2005; Bjorå 2008; Elden 2010). Thus, even after the transfer of *C. neghellense* (Fig. 1B) to *Anthericum*, the generic delimitation is not satisfactory and will have to be studied further in a broader geographic and taxonomic context.

Clade A (Fig. 3a, b) comprises the African species of the genus *Anthericum* that are near-endemic to the Horn of Africa. Only *A. corymbosum* Baker (1877: 71) extends south to northernmost Tanzania. *Anthericum angustifolium* Hochstetter (1850: 332), *A. corymbosum* and *Anthericum* sp. constitute a clade with high support (ITS PP 1/JK 100; pDNA PP 1/JK 94). The two former are morphologically distinct: *A. angustifolium* has a completely reduced peduncle and rachis and long-pedicellate flowers in a pseudo-umbel and *A. corymbosum* has a well-developed peduncle and flowers in a racemose inflorescence. The former appears to be better adapted to heavy grazing by keeping flowers and fruits close to the ground. The two species show a vicariant distribution pattern in Ethiopia: *A. angustifolium* widely distributed in the central, northern and south-western Ethiopian highlands (Tigray, Gonder, Welo, Shewa and Gamo Gofa floristic regions; Fig. 2B) and in Eritrea; *A. corymbosum* distributed in the southern and eastern parts at somewhat lower elevations (Harerge, Bale and Sidamo floristic regions; Fig. 1B). *Anthericum* sp. from the Bale floristic region has only one flower per plant on a long pedicel, and might represent a depauperate *A. angustifolium*. However, more material is needed to conclude on this.

The strongly supported sister to the *A. angustifolium/A. corymbosum* clade (ITS PP 0.99/JK 77; pDNA PP 1/JK 99) is *A. jamesii* Baker (1898: 490), distributed in the arid triangle between SE Ethiopia, NE Kenya and SW Somalia. *Anthericum neghellense,* a narrow endemic in southern Ethiopia, is in turn sister to all the other African *Anthericum* species. Given the distribution of the taxa in clade A, speciation within the African *Anthericum* has probably taken place in the Horn of Africa. Taxa in clades B and C (Fig. 3a, b) were earlier referred to the genus *Anthericum* sensu Obermeyer (1969), defined by capsules with horizontal ridges and irregularly folded seeds. These taxa were, however, transferred to the genus *Chlorophytum* by Kativu & Nordal (1993). In our gene trees, these taxa do not constitute a monophyletic group, but as the two clades are part of a larger unsupported polytomy, monophyly cannot be ruled out. A sister relation between clades B and C does not gain much support from morphology, but both clades differs significantly from Clade A. Taxa of clade A have simple inflorescence nodes and no pedicel joint, whereas taxa of clade C have complex inflorescence nodes and pedicel joints (as the remaining clades D–G).

The E. African endemic *C. suffruticosum* Baker (1878: 326) (one of the very few woody species in the genus) and the widespread polymorphic *C. subpetiolatum* (Baker 1876: 302) Kativu (1993: 64) (Fig. 1C) are supported as sister species by both molecular datasets (Fig. 3a, b: clade B). Support for monophyly of different accessions of *C. subpetiolatum* across geographic distance (Ethiopia to Zambia; Fig. 3a) suggest this is a good species (ITS PP 1/JK 99).

The species of clade C, which receives strong support in both trees (Fig. 3a, b; PP 1/JK 100) are characterized by flowers with a zygomorphic androecium and pinkish coloration. In this clade, *C. cameronii* (Baker 1876: 314) Kativu (1993: 62) is the only Ethiopian representative, the clade otherwise comprising several species, distributed to the south and west of the Horn of Africa (Bjorå 2008). *Chlorophytum cameronii* is rare in Ethiopia, only found in lowlands close to the Sudanian border (Wellega and Illubabor floristic regions), suggesting it might have entered the Ethiopian flora from the south or west.

Several highly supported clades correspond well with the morphological groups introduced by Bjorå (2008) (Fig. 3a, b: clades D–G). The species comprising the strongly supported clade D (Fig. 3a, b) are characterised by a distichous leaf arrangement, a trait otherwise only found in clade C. Clade D divides into three subclades. Monophyly of *C. affine* is not supported by our molecular analyses; as the two varieties, var. *affine* and var. *curviscapum*, occur in different subclades. The two accessions of Ethiopian var. *curviscapum* represent a sister group to *C. humifusum* (ITS only; Fig. 3a), whereas *C. affine* var. *affine* (Fig. 1D) comes out in another subclade as sister to *C. pubiflorum* Baker (1876: 329) and *C. polystachys* Baker (1878: 326), all from Zambia. *Chlorophytum affine* var. *affine* is delimited from *C. affine* var. *curviscapum* by broader leaves (11–22 mm vs. up to 9 mm) and by an erect, not basically curved peduncle. Material of *C. affine* from the Horn of Africa was earlier referred to *C. tordense*, which was sunk into *C. affine* var. *curviscapum* in FTEA by Nordal *et al.* (1997). Our results show that *C. affine* var. *curviscapum* is not conspecific with *C. affine*, and the name *C. tordense* should accordingly be re-instated (since when recognised at species level, *C. tordense* has priority to *Anthericum curviscapum*, the basionym of var. *curviscapum*).The third subclade consists of two Ethiopian accessions of the morphologically distinct *C. pendulum* Nordal & Thulin (1993: 273).

Chlorophytum tordense and *C. humifusum* grow in disturbed, open and grazed areas and have developed prostrate inflorescences, a putative strategy to avoid grazing on flowers and fruits. *Chlorophytum pendulum*, on the other hand, with erect scapes and large hanging, pyramidal-shaped capsules, grows in more densely vegetated areas, without very heavy grazing. *Chlorophytum humifusum* and *C. pendulum* are near-endemics extending their distribution to southern Ethiopia and northern Kenya. The distribution of *C. tordense* outside Ethiopia is difficult to assess as its relation to *C. affine* var. *curviscapum* is not clear.

Clade E is strongly supported by all our molecular data (Fig. 3a, b), but only when *C. zavattarii* is excluded from the ITS analysis (not shown). This clade is also morphologically well-defined; all members have paniculate inflorescences and spongy roots without tubers. They are all relatively tall plants. Morphologically, *C. zavattarii* clearly belongs to clade E, and in the nuclear tree (Fig. 3a), it is sister to *C. viridescens* in the same clade. However, in the plastid tree (Fig. 3b), *C. zavattarii* is sister to *C. pterocarpum* Nordal & Thulin (1993: 274) (in clade F), which does not belong to the paniculate spongy-rooted group. *Chlorophytum zavattarii* and *C. pterocarpum* occur in the same area and habitat, while *C. viridescens* Engler (1895: 140) grows further south in tropical East Africa. Thus, the molecular similarity of *C. zavattarii* to *C. pterocarpum* in the plastid tree might be a result of hybridization and chloroplast capture. The recently described *C. pseudocaule* (Fig. 1D, Tesfaye & Nordal 2007) was, based on morphology, hypothesized to belong to "the paniculate spongy-rooted group". This is corroborated by our molecular data (Fig. 3a, b). This species is a narrow endemic from a seasonally wet meadow in the Wellega floristic region, close to the Sudanian border. The other species in clade E occur further south and particularly in the Zambesian vegetation region (sensu White 1983). The presence of two endemic species in clade E, namely *C. zavattarii* and *C. pseudocaule*, suggests that speciation may have taken place in the Horn of Africa.

Clade F is strongly supported by all molecular data (Fig. 3a, b), but only when *C. zavattarii* is excluded from the plastid tree (not shown). Morphologically, species within clade F share the traits of having one-flowered nodes (except *C. longifolium* Schweinfurth (1867: 294), which has multi-flowered nodes), triquetrous capsules, and completely flat seeds, densely stacked in the flat compartments of the fruit. Those species formerly referred to a separate genus, *Dasystachys* Baker (1898: 490) (see indication in Fig. 3a, b), form a poorly supported subclade within clade F in the nuclear tree (Fig. 3a) but receive no support from the plastid data (Fig. 3b). *Dasystachys*, which was sunk into *Chlorophytum* by Marais & Reilly (1978), is characterized by having bell-shaped, closed flowers in a spicate inflorescence. Only two of the previous *Dasystachys* taxa, *C. longifolium* and *C. africanum* (Baker 1875: 160) Engler (1893a: 470) var. *silvaticum* (Dammer 1912: 365) Meerts (2012: 385) (treated as *C. silvaticum* in FEE and FTEA, see Meerts & Bjorå 2012), occur in Ethiopia, although none of them are very common. *Chlorophytum longifolium* is found in northwest Ethiopia, close to the Sudanian border (Tigray and Gonder floristic regions), from where the species originally was described. It is otherwise widely distributed in the Sudano-Zambezian region (sensu White 1983) south to Botswana and Namibia. *Chlorophytum africanum* var. *silvaticum* is only recorded from the southernmost part of Ethiopia (the Sidamo floristic region), but is otherwise widely distributed south to Zimbabwe and Mozambique. Given their current distributions, we suspect that these two species have originated further south and reached Ethiopia from the west or south. Unfortunately, no Ethiopian material could be obtained for the present study

The "non-*Dasystachys*" members of clade F (*C. somaliense* Baker, in Baker & Engler 1893: 469 and *C. pterocarpum*) do not display bell-shaped, closed flowers. *Chlorophytum somaliense* (Fig. 1F), widespread on the Horn of Africa (including Kenya), is a large plant, with a general appearance resembling the *Dasystachys* taxa. It has, however, conspicuous flowers with reflexed tepals and an exerted, zygomorphic androecium and clear herkogamy. We hypothesize that, in the course of evolution, this difference might have been pollinator driven. *Chlorophytum pterocarpum*, a narrow endemic in Ethiopia (the Sof Omar area in the Bale floristic region), is a small plant with small, inconspicuous, open flowers. It appears to be a dwarfed sister to *C. somaliense*, adapted to arid conditions. *Chlorophytum pterocarpum* is possibly autogamous as insect attractants appear to be lacking. It seems likely that species divergence among the "non-*Dasystachys*" members of clade F have taken place in Ethiopia.

Clade G is supported by all molecular data (ITS PP 1/JK 72; pDNA PP 1/JK 59) and corresponds to what has been referred to as "*Euchlorophytum*" (Bjorå 2008). This group is defined by having a basic chromosome number of x = 7, contrasting the other *Chlorophytum* species with x = 8. Representatives of the genus *Chlorophytum* outside the *Euchlorophytum* clade are, with few exceptions, found in savanna grassland, bushland and woodland. The members of the *Euchlorophytum* clade seem to have adapted to more shady forest environments, including riverine forests.

Clade H, which might be denoted the *C. gallabatense* complex, is supported by the ITS data (Fig. 3a, PP 1/JK 74) but receives less support by pDNA (Fig. 3b, PP 0.96). In the pDNA tree (Fig. 3b), clade H also includes *C. geophilum* (in clade J of the ITS tree; Fig. 3a). Also without *C. geophilum*, the *C. gallabatense* clade, distributed from Ethiopia west to Senegal and south to Zimbabwe, is rather heterogeneous molecularly, and two subclades are evident: (1) two Ethiopian accessions of *Chlorophytum* sp., and (2) three Ethiopian accessions of *C. gallabatense*. The two Zambian accessions of *C. gallabatense* do not group with the Ethiopian accessions (Fig. 3a, b). Representatives of the *C. gallabatense* clade created, as mentioned, problems when writing up the FEE (Nordal 1997). It was questioned in the flora whether specimens, here referred to *Chlorophytum* sp., represents shade forms of *C. gallabatense*, or whether they represented forms of the southern African *C. comosum* complex. Our molecular data suggest that *Chlorophytum* sp. belongs in the *C. gallabatense* complex. Yet, the two accessions of *Chlorophytum* sp. group with high molecular support (ITS PP 1/JK 95; pDNA PP 1/JK 94), suggesting it represents a distinct evolutionary lineage that also gains support from the morphological investigations; *Chlorophytum* sp. is distinct from *C. gallabatense* in several independent morphological traits (Table 2). The morphology of the inflorescence indicates that mechanism of seed dispersal differs between the two: *Chlorophytum* sp. releases the seeds on the ground at some distance from the mother plant due to the fairly long and lax inflorescence, whereas *C. gallabatense*, with its stiff erect peduncle, displays all traits of a typical ballist. In addition, the two species differ in the morphology of the seed testa. *Chlorophytum* sp. should be recognised at the level of species and is described below as *C. mamillatum* Elden & Nordal (due to the small nipple-like projections of the testa cells; Fig 4A). This action renders the species *C. gallabatense* paraphyletic in the molecular trees (Fig. 3a, b: clade H). Moreover, the molecular results indicate that the species *C. gallabatense* is heterogeneous and that Zambian specimens represent one or more distinct evolutionary lineages that may deserve the rank of species. The *C. gallabatense* complex should be investigated further with a broader geographic sampling.

As mentioned above, the plastid versus nuclear trees are ambiguous when it comes to the phylogenetic placement of *C. geophilum* (Fig. 3a, b: clades J and H, respectively). *Chlorophytum geophilum* is only found within the Illubabor and Gonder floristic regions in the extreme west of Ethiopia, but is otherwise widely distributed in savanna areas in Africa, west to Burkina Faso, and south to Malawi and Zambia. The grouping of Zambian and Ethiopian accessions indicates monophyly of the species, but further investigations are needed to confirm this and also its relations within the "*Euchlorophytum*" clade.

Monophyly is supported also for the heterogeneous *C. blepharophyllum* complex (cf. Bjorå 2008), but only in the ITS tree (Fig. 3a, PP 1/JK 93). In Ethiopia, *C. blepharophyllum* (Fig. 2B) is found in the western parts of Tigray, Gojam, Wellega and Illubabor floristic regions. Otherwise, *C. blepharophyllum* is widespread in tropical Africa, from Ethiopia west to Senegal, through Central and East Africa, south to Angola, Zimbabwe, and Mozambique. Taxon delimitation in the *C. blepharophyllum* complex should be investigated further with a broader taxonomic and geographic sampling.

Clade I, a well-supported subclade of clade G (PP 1/JK 99), comprises three Ethiopian species, the near-endemic *C. tetraphyllum* Baker (1876: 328) and the narrow endemics, *C. herrmannii* and *C. serpens* (Fig. 3a, b). This clade is characterized by plants with more or less prostrate leaves and inflorescences, which might have evolved as a strategy to avoid grazing. *Chlorophytum tetraphyllum* was originally described from Yemen by the younger Linnaeus, but has its main distribution in the Ethiopian highlands. Sebsebe & Nordal (2010) proposed that *C. tetraphyllum* should be transferred to *Anthericum*. The morphological basis for the proposed transfer was that *C. tetraphyllum* apparently lacks pedicel articulation (Nordal 1997). However, the peduncle and rachis are otherwise so reduced that the diagnostic character of complex nodes or not is difficult to evaluate. The new combination, *Anthericum tetraphyllum* (Linnaeus fil. 1782: 200) Nordal & Sebsebe (2010: 133), was proposed. The name was, however, not validly published, as an exact reference to the basionym *Scilla tetraphylla* Linnaeus fil. (1782: 200) was not included. Our results clearly show that the taxon belongs in *Chlorophytum*.

Chlorophytum herrmannii and *C. serpens* are only known from a few populations in the far west of Ethiopia close to the Sudanian border (Wellega and Gojam floristic region). In this clade, speciation appears to have taken place locally in W Ethiopia.

The two accessions of the Ethiopian endemic *C. ducis-aprutii* Chiovenda (1929: 370) (Fig. 1I) constitute a well-supported subclade within clade G (Fig. 3a, b; ITS PP 1/JK 98; pDNA PP 0.91/JK 57). Agegnehu *et al.* (2012) documented that the chromosome number of this species is 2n = 30, and as such represents x = 15 within the otherwise homogenously x = 7 "*Euchlorophytum*" clade. The most probable interpretation is that the species is an allopolyploid between an x = 7 and an x = 8 taxon. Based on morphology, we suspect that *C. macrophyllum* Ascherson (1867: 294), widespread in Ethiopia, might be the putative x = 7 parent. When it comes to the putative x = 8 parent, the size of the plant (60–200 cm high), the spongy roots without tubers, the bracteate peduncle and the branched inflorescence might indicate a member of "the paniculate spongy rooted group" (cf. Fig. 3a, b: clade E). Nordal *et al.* (1990) published the chromosome number 2n = 32 for *C. ducis-aprutii*. However, the preparation was not optimal and the result could, in hindsight, be interpreted both as n = 15 or n = 16. The hypothesized allopolyploid origin of *C. ducis-aprutii* might be further illuminated by various molecular approaches. In FEE, a somewhat deviating form of *C. ducis-aprutii* was recorded from Eritrea. The taxonomic belonging of this form needs further investigation.

Our molecular results clearly show that *C. comosum*, as circumscribed in FTEA (Nordal *et al.*1997), is highly heterogeneous (Fig. 3a, b; subclade J and K). The species was described from South Africa by Thunberg already in 1794, and the name is accordingly attached to the plants of clade K. This means that the East African *C. comosum* in clade J, and probably also the Ethiopian plants (unfortunately, material not available for molecular analyses), should be referred to a different species. Genetic diversity in relation to geographic distance should be further analysed in *C. comosum*.

The two species *C. macrophyllum* and *C. filipendulum* Baker (1878b: 260) have often been confused in herbaria and literature (e.g., Nordal *et al.* 1997). *Chlorophytum macrophyllum* is described from the Tigray floristic region in Ethiopia and is otherwise widely distributed in tropical Africa from Ethiopia west to Sierra Leone and south to Mozambique, growing in dense woodland and transitional vegetation woodland/forest within Sudano-Zambezian vegetation (White 1983). *Chlorophytum filipendulum* is a typical Guineo-Congolean (sensu White 1983) rain forest species; in Ethiopia only found in the south-west (Kefa floristic region). Morphologically the two species differ in flower traits. *Chlorophytum macrophyllum* has large showy flowers (tepals 9–15 mm long) and anthers longer than the filaments. *Chlorophytum filipendulum* has inconspicuous flowers (tepals 5–7 mm long) and anthers shorter than the filaments. Due to low resolution, our molecular results do not clarify the relation. The delimitation within this complex needs further attention.

Biogeographical aspects

The near-endemic species of *Anthericum* and *Chlorophytum* in Ethiopia fall mainly within two areas (Fig. 2B). The first of these evolutionary hotspots is found in the lowland south and east of Bale Mountains, i.e. the Sidamo and Bale floristic regions, (some also extending into adjacent parts of Kenya and Somalia) and includes the following six species: *A. neghellense, C. bifolium, C. humifusum, C. pendulum, C. pterocarpum*, and *C. zavattarii.* This putative centre of endemism was already recognized by Nordal *et al.* (2001). The following three species occur within the same area, but extend further east to the Harerge floristic region: *A. jamesii, C. ducis-aprutii* and *Chlorophytum* sp. (i.e. *C. mamillatum*; described below). Speciation in this southern to eastern group of endemics has taken place in several clades, indicating that the relative high number of endemics in the area is not due to radiation in one particular clade, but is spread across the two genera. The areas, where the above mentioned endemic species are distributed, are characterised ecologically by bimodal rainfall with the first peak between September and November and the second between March and May. The vegetation is dominated by *Acacia*-*Commiphora* woodland and bushland. The *Acacia*-*Commiphora* woodland is divided into two subtypes. The areas where the endemic *Anthericum* and *Chlorophytum* species occur belong to the subtype *Acacia-Commiphora* woodland and bushland proper ("ACB" of Friis *et al.* 2010), characterized by drought-resistant trees and shrubs.

The second evolutionary hotspot is found in the lowland of Gojam and Wellega, close to the Sudanian border, and includes the following endemic species: *C. herrmannii, C. pseudocaule* and *C. serpens.* This centre of endemism was also recognized by Sebsebe *et al.* (2005). Speciation seems to have taken place in both clades E and I (Fig. 3a, b). The region is characterised by unimodal rainfall between May and September. The vegetation is dominated by *Combretum*-*Terminalia* woodland and wooded grassland (Friis *et al.* 2010). This vegetation type is characterized by small to moderate- sized trees with fairly large deciduous leaves and a well developed tall grass stratum. The grass stratum burns during the dry season and the vegetation in general is adapted to fire.

The two remaining near-endemics, *A. angustifolium* (clade A) and *C. tetraphyllum* (clade I), have a fairly wide distribution in the Ethiopian highland and often grow in heavily grazed, even over-grazed, areas. The area with the highest density of endemics is, as mentioned, the lowland surrounding the Bale Mountains. During the Quaternary climatic fluctuations, plants might have found rather stable niches by moving up and down the mountain according to changes in particular rainfall.

When it comes to the western center of endemicity, Sebsebe *et al.* (2005) suggested that the complex topography and the relatively reliable orographic rain on the western Ethiopian escarpment, together with the hinterland of deep river valleys, provided small refugia during the periods of adverse climatic conditions. This may have secured niches where species could survive unfavourable periods. The best conditions for such niches are likely to have been in the most topographically and geologically complex areas in the lower reaches and at the mouth of the biggest river system in western Ethiopia, the gorges of the Blue Nile River and its tributaries.

Taxonomic conclusion

In addition to understand the phylogenetic sister group relations of the Ethiopian endemic species of *Chlorophytum*, as discussed above, an aim of the paper was to sort out the variation pattern in the *C. gallabatense* complex, and particularly to establish the taxonomic status of the so-called "shade form". Based on both molecular and morphological evidence, this form, herein mostly referred to as *Chlorophytum* sp., deserves taxonomic recognition at the level of species and is formally described below.

Chlorophytum mamillatum Elden & Nordal, *sp. nov*. (Fig. 5)

Species nova C. gallabatensi *affinis, sed eo differt tuberibus secus axem radicis non in ramis lateralibus dispositis; inflorescentiis laxis prostratis; floribus albis non viridibus; testis periclinalibus cellularum mamillatis.*

A species related to *C. gallabatense*, differing by having root tubers along the root axis, rather than on lateral branches; lax, prostrate inflorescences, not stiffly erect; white flowers, not greenish; and nipple-like projections on the periclinal seed cell walls.

Type:—ETHIOPIA. Bale floristic region: 10 km N of Dolo Menna (Masslo) on the road to Goba, 6º25’ N 39º44’E, 1500 m of elevation, 25 October 1984, *I. Friis, M.G. Gilbert & K. Vollesen 3459* (holotype ETH!; isotype K!).

Perennial with short rhizome, roots ca. 2 mm in diameter, with distal tubers, 20–40 × 5–10 mm. Leaves rosulate, strap-shaped, 150–600 × 15–30 mm, glabrous with narrow hyaline margin. Inflorescence paniculiform, length (including peduncle) 250–500 mm with 2–7 branches. Bract supporting branches up to 12 mm long, usually shorter; bracts supporting flower nodes up to 3 mm long, with dark brown margin. Number of flowers per node 1–3(4). Pedicel length 3–5(–7) mm, with a joint disposed from the middle to upper part, slightly papillose below the joint (at least in fresh material). Flowers star-shaped, white with greenish apex; tepals free, lanceolate, 4–5 × 1.5–2 mm; filaments filiform, 2–3 mm long, slightly papillose, white; anthers 1–2 mm long, yellow; style 4–5 mm long, bent with a minute stigma. Capsule triangular in cross section, papillose, ca. 4 × 6 mm; seeds 3 or 4 per locule, diameter 2 mm, flat and irregularly folded, periclinal walls of epidermal cells conical and outdrawn to a distinct papilla (Fig. 4).

Distribution: —The Bale and Harerge floristic regions, Ethiopia (Fig. 2B).

Additional specimen examined: —ETHIOPIA. Bale floristic region: 10 km N of Dolo Menna (Masslo) on the road to Goba, 06°25’ N, 39°44’ E, 1500 m elevation, 25 October 1984, *I. Friis, M.G. Gilbert & K. Vollesen 3459* (ETH, K); Harenna Forest, 14 km on Dello Mena–Goba road, 06°27’ N, 39°44’ E, 1560 m elevation, 11 June 1986, *M. Tadesse 4671* (ETH); Dello Awraja in Harenna forest, ca. 2.2 km from turn-off from Shisha River, ca. 20 km on Dello Mena–Goba road, 06°29’ N, 39°452’ E, 1530 m elevation, 13 August 1986, *M. Tadesse 5291* (ETH); Harenna Forest near Yadot River, 1625 m elevation, 2 May 1990, *L. Nogatu* and *M. Tadesse 9076* (ETH); Welmel River, Fenkel Kebale, 06°26.576’ N, 39°39.153’ E, ca. 1500 m elevation, 15 December 2007, *M. Elden, I. Nordal, T. Awas & S. Demissew 9* (ETH, O). Harerge floristic region: near Graua 9°08’ N 41°55’E, 1750–1900 m elevation, 9 August 1975, *M.G. Gilbert 4019* (K); Harar–Jijiga road, 13 km, 1550 m elevation, 17 August 1975, *M.G. Gilbert & M. Thulin 41* (K); Uadendeo Plateau, 36 km ESE of Harar on the road to Jijiga, 9°04’ N, 42°23’ E, 1520 m elevation, 17 August 1972, *W. Burger 2074* (K); East of Gara Gora, 9°00’ N, 42°18’ E, 1220–1340 m elevation, 28 July 1963, *W. Burger 3050* (K).

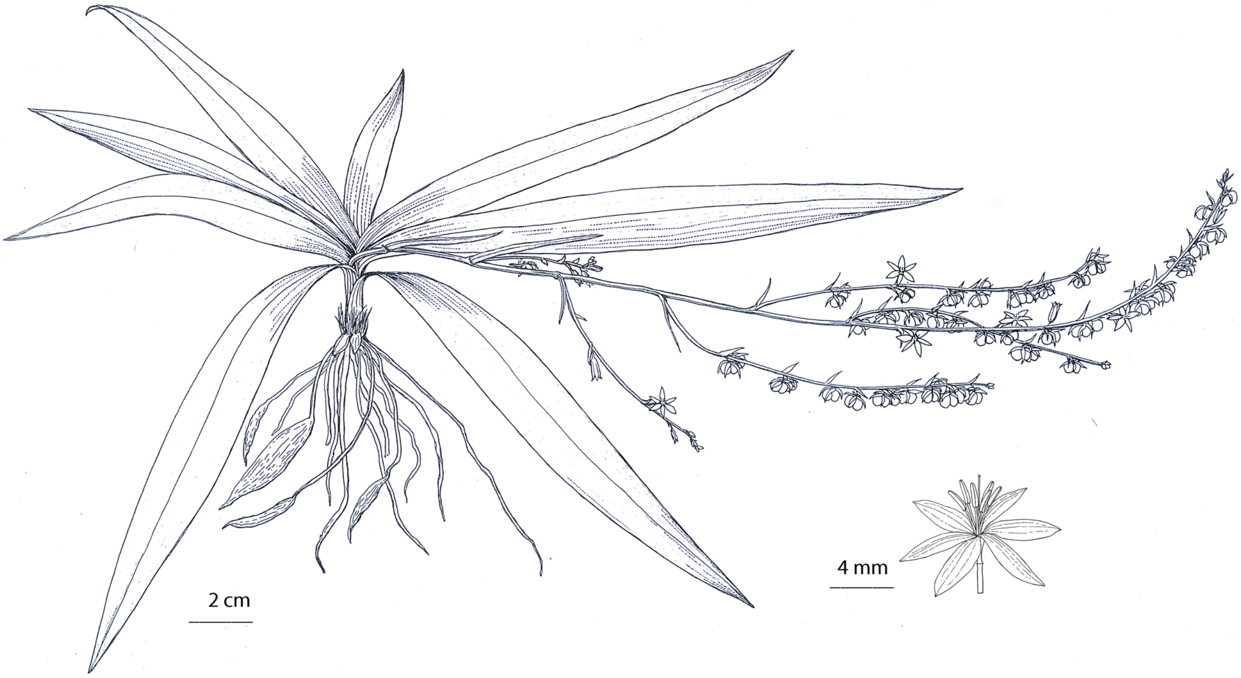


FIGURE 5. *Chlorophytum mamillatum* based on *Gilbert & Tulin 41* (K). A. Habit. B. Detail of flower. Artist: Svetlana Voronkova.

Acknowledgements

We thank the curators in BRLU, ETH, K and WAG for the loan of material, in particular Prof. P. Meerts and Dr. J. Wieringa for providing samples collected in silica gel. We also thank Lisbeth Birgitte Thorbek and Cecilie Mathiesen for assistance with molecular analyses, Melinka Butenko for assistance with scanning electron microscopy and Svetlana Voronkova for the line drawing. We thank the editor and two anonymous reviewers who improved the quality of this paper.

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